Title: IMMUNOGENIC PILI
PRESENTING FOREIGN PEPTIDES,
THEIR PRODUCTION AND USE
Inventor(s): Peter O'Hanley et al.
DOCKET NO.: 050939/0104

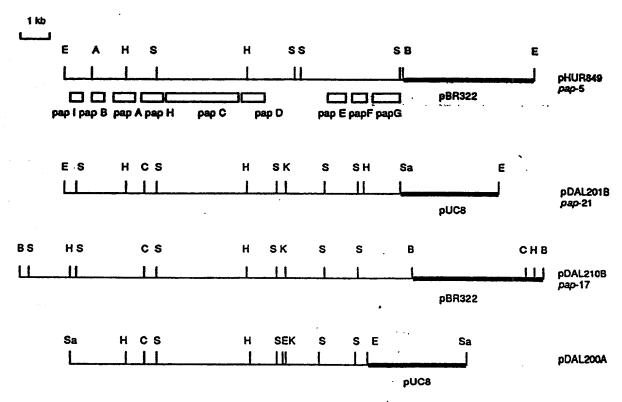


Figure 1. Genetic and physical map of recombinant plasmids pHUR849, pDAL201B, pDAL210B, and pDAL200A. The locations of of the pap genes shown as open bars. Restriction site abbreviations: A, Apa I; B, Bam HI; C, Cla I; E, Eco RI; K, Kpn I; Sa, Sal I; and S, Sma I.

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3		120 Hall Hall Clark St. 120 Hall St. 120 Hal	A C P P P D M S L P V M C P P P D M S L P P V M C P P P D M S L P P V M C P P P P D M S L P P V M C P P P P D M S L P P P V M C P P P P D M S L P P P V M C P P P P D M S L P P P V M C P P P P D M S L P P P V M C P P P P D M S L P P P V M C P P P P P D M S L P P P V M C P P P P P D M S L P P P V M C P P P P P D M S L P P P V M C P P P P P P D M S L P P P P P P P P P P P P P P P P P P	120
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30	AGTCAGGGGGGGGGGGGGTTTCGTGATTCCGGATAAGGGGGACTTTCGATGGCGTCGG	360 301	ATTOMOGRAPH COTTENT TO THE TOTAL OF THE TOTAL TO	360
36	GOTGANAGOCCOGATAAGTTAATTAATCOGGTCAGCAAAAGGCATTAATCTGCAGATA	420 361	GOTGAMACCCGARACTTRATTCRICCOCCOCCOCCOCCAMITANTCTCCCAARA	430
\$ *	CTCATOTOROGCAAATATTCCCCGGCACAAAACATAAAGAAAAAAAAAA	400 421	GCTUATUCCAGOGAAATATTGCCGGCAAGGAAACCATTGACGAAAACAATTGACGAAAAAAAA	9
\$	GOTALIDACAGOCOCOCATACACCOCATATION CALANCOCALANAMONTOLA GINERE ALDYTELE IVENIVENION CONTRACTORA	540 481	GOTATUMANGAGCTOCATACHOCCTCACAATTGTGCGAAACAAAAAACTTGAA G N B B A L D Y T L R I V R N G K K L E	\$40
5 ,	OCCOGARATATATACTOTOCTOCOGATTCCOCOTCGATATACACTCA SAS A G H Y F A V L G F R V D Y E	198	OCCOGNANTATIOCCOTOCTOCONATICCOCOTCANTANGACTON SON	
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	ATCACACTOCCATTOCCATTACTATATACCATGATACTACATCCATCATACTATACTATACTATACTATACTATACTATACTATACTATACTATACTATACTATACTATACTATACTATACTATACTAC		ANGAGLOCICATION TOTATA TATA TOTATO TO	3
3	TTGCCGGTCCGTTCCTCCTCCCTCCTCCTGATACTGGGAAAAAACACACAC	120 61	-22 TITOCCOOPCOOTITICITICOCCOCATOCCTTCCTGAATACACACACACACACACACACACACACACA	120
2	CTATUTOGRACOCCACCTOCTTTCATCOTCACCTCCACCTCCTCTCTCT	121	-1+1 GINTUGIOGACOGCIACITITICAÍGGIAGACATGICAACCIACAACTACTAG V W W D G R A A F H G E V V R P A C T L	180
5 .	CHARLES AND I I D M G & T P V R D L Q	240 . 161	GCGATGGAAGGCCTGGCAATTATGGAAATGGGGAAACCCGGGTTGGGATTTACAGAA WAA AA BAAAAAAAAAAAAAAAAAAAAAAAAAAA	
2	ATIGETTTTCCCCACCTCAAAAATTCACCTCCCCCCCACAAAATTAAC	300 241	ATTOSTITITICOGACCTGAAAAAATCAGCCTCCGGCTCAGAACTGTGAATTAAC N G F 8 G P E R K P S L R L R N C B P M	300
301	ASTEAGGEACCTTTCTCTGATTCCCGATAAGGTGACTTTCCATGGCGTCCGG	360	ASTCAGOGIGGGAACCTTTTCTCTGATTCCCGGATAAGGGGACTTTCGATGGTGTCCGG	360
361	GOTGARACCCCCATARATTRATCCCCTCRGCCATARACCCATARATTCCCACATA	420 361	GOTGAAACCCCCCATAACTTAATTTATCCCGTCAGCCAAAAGCCATTAATCTGCAGATA G B T P D K F N L S G Q A K G I N L Q I	62
123	CCTCATCCCACCCAAATTCCCCCCCCCAAACTATCCCTCCAATACCATTCACC	480 421	OCTOATGCCAGGGAATATTGCCCGCAGGAAAGTAATGCCTGCAATACCATTGAGGAA DA R G W I A R A G K V M P A I P L T	0
Ş	GOTALTANDAGOCTOSTATACACTCASAATTOTOTOTALAACTTANA G N E E A L D Y T L R I V R N G K K L E	240	SACAATTGTGGGAAAC	240
3	A C N Y F A V L G F R V D Y E	541	OCCOCANATATIVIC COCICOCATIVICACION SEE	

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Figure 2. DNA sequences of pHUR849 (A), pDAL2018 (B), pDAL210B (C), and pDAL200A (D) papH structural genea. The nontranscribed DNA strand for each clone shown. Numbering is from the 5' end . The deduced amino acid sequence for the correct frame is shown below each DNA strand. The first amino acid of the mature protein is +1. Stop codon is marked with asterisks. The restriction sites for Cla I (ATGGTA), and Sma I (CCCGGGS), are underlined once, respectively.

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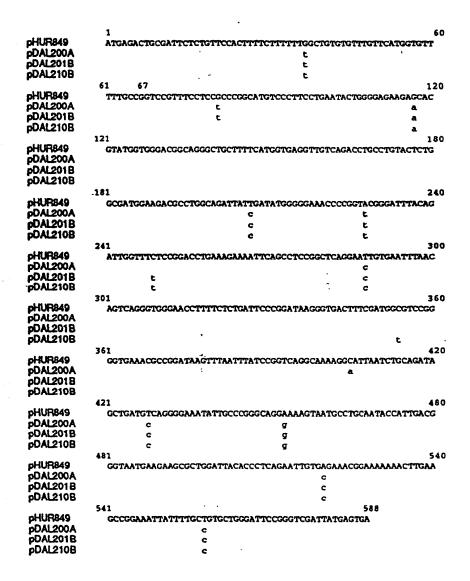


Figure. 3. Comparison of deduced nucleotide sequence of papH genes pHUR849, pDAL200A, pDAL201B and, pDAL210B. The nucleotide(nt) identities, compared with the deduced sequence of PapH nt sequence papH gene of pHUR849 (upper case), are indicated by blank space, nt differences for the PapH genes of pDAL201B, pDAL210B and, pDAL200A, are shown with the corresponding single letter at code (lower case), respectively. Numbering is from the 5' end. The first at of coding for the leader sequence is numbered 1, and the first nt coding for the mature protein is numbered 67.

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-1 +1 18

MRLRFSVPLFFFGCVFVHGVFAGPFPPPGMSLPEYWGEEH

19

VWWDGRAAFHGEVVRPACTLAMEDAWQIIDMGETPVRDLQ
59

NGFSGPERKFSLRLRNCEFNSQGGNLFSDSRIRVTFDGVR
138

GETPDKFNLSGQAKGINLQIADVRGNIARAGKVMPAIPLT

173

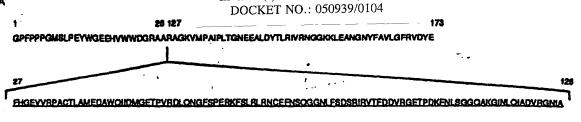
GNEEALDYTLRIVRNGKKLEAGNYFAVLGFRVDYE

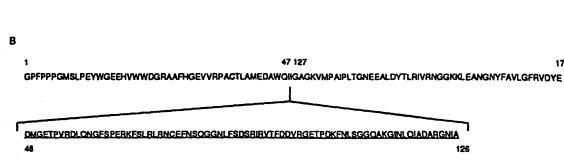
Figure. 4. Comparison of deduced amino acid sequence of papH genes pHUR849, pDAL201B, pDAL210B and, pDAL200A. The vertical arrow indicates the postulated cleavage site for the signal peptidase. The amino acid (aa) identities, compared with the deduced sequence of PapH protein of pHUR849 (upper case), are indicated by blank space, as differences for the PapH proteins of pDAL201B, pDAL210B and pDAL200A, are shown with the corresponding single letter as code(lower case), respectively. The first as of the leader sequence is numbered -22, and the first as of the mature protein is numbered +1.

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Figure. 5. Comparison of deduced amino acid sequence of papH gene deletion mutants pHUR849-5(pap-5), pDAL201B(pap-21), pDAL210B(pap-17) and, pDAL200A(pap 200A). The deduced amino acid sequence of each of the finial constructs is shown, (A) pHUR849-5 and, (B) pDAL201B, pDAL210B and, pDAL200A which are identical to each other. The amino acid identities of the proteins are upper case letters. The first amino acid of the mature fusion protein is numbered 1. The underlined sequence indicates the amino acid residues deleted from the mature fusion protein of each strain.